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WO 94/25477 A1 WO 93/24511 A1 WO 92/20703 A1
Science 1991,254,1497-1500 J.Am.Chem.Soc. 1992, 114(5),1895-1897 Proc.Natl.Acad.Sci.USA 1993, 90(5), 1667-1670

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UK CL (Edition N) C3H HB3 HB5 HHX2 , G1B BAH
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(54) Nucleic acid analogues with a chelating functionality for metal ions

(57) A nucleic acid analogue comprises a polymeric strand including a sequence of ligands bound to a backbone made up of linked backbone moieties and has a moiety capable of binding at least one metal ion by chelation. The analogue is capable of hybridisation to a nucleic acid of complementary sequence. A solution of the analogue may readily be concentrated by capturing the analogue from solution on to a solid support having chelatable metal ions or may readily be labelled by a label comprising a chelatable metal ion as a label or having a label linked to a chelatable metal ion. The backbone may comprise a polyamide, polythioamide, polysulphinamide or polysulphonamide, preferably poly(amino acid) The chelator is preferably an amino acid, oligopeptide (especially $(His)_5$) or polycarboxylic acid (especially EDTA).

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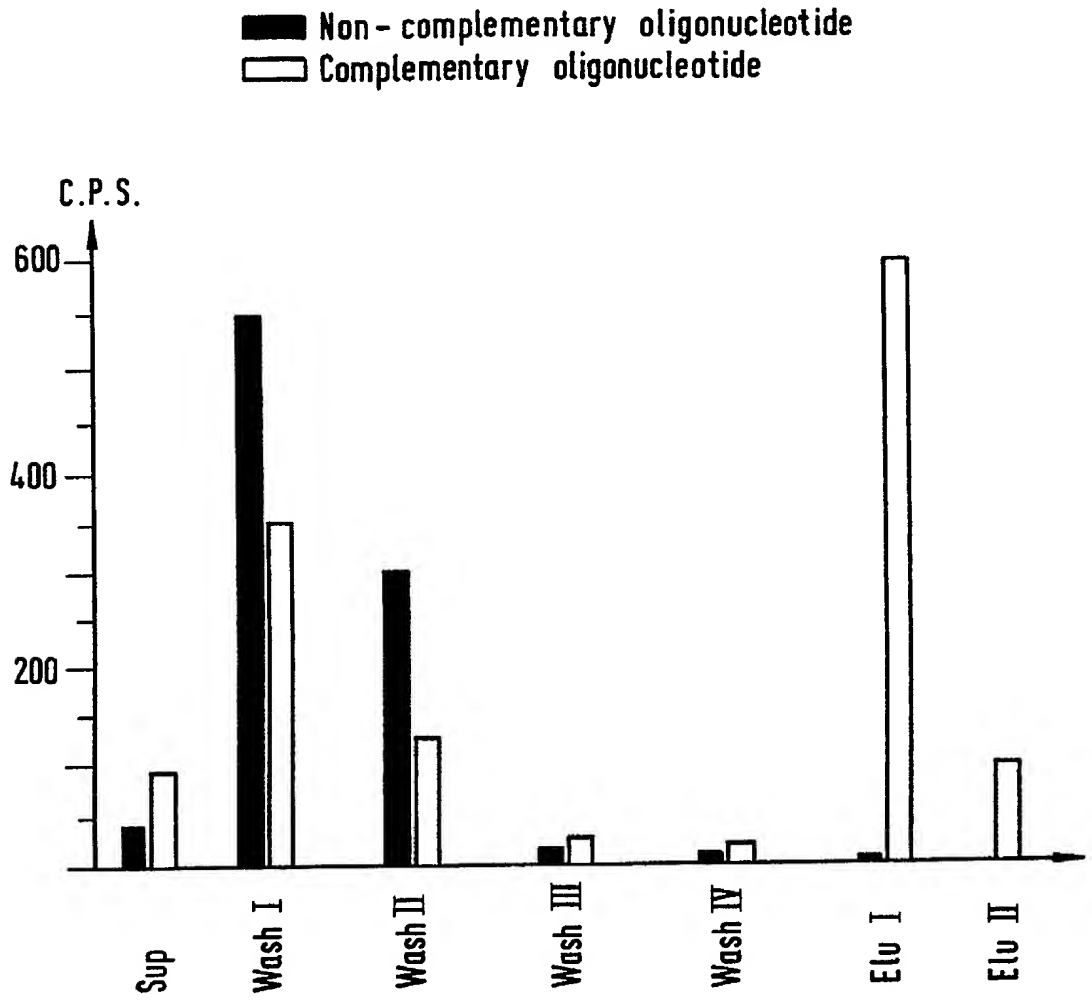
At least one drawing originally filed was informal and the print reproduced here is taken from a later filed formal copy.

Fig. 1


Non - complementary oligonucleotide
Complementary oligonucleotide
C.P.S.
600
400
200
Sup
Wash I
Wash II
Wash III
Wash IV
Elu I
Elu II

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NUCLEIC ACID ANALOGUES WITH A CHELATING FUNCTIONALITY

The present invention relates to nucleic acid analogues having a chelation functionality, to their uses in assay procedures, to methods of capturing them to solid supports and to methods of concentrating solutions of them.

Nucleic acid analogues having important new utilities in assay procedures and in the field of diagnostics have been described in WO 92/20703. These nucleic acid analogues had a number of new properties making them of special importance in the field of diagnostics as well as in the field of antisense therapeutics.

They typically feature a polyamide backbone bearing a sequence of ligands which are nucleic acid bases. The analogues described there have the property of hybridising with great specificity and stability to natural nucleic acids of complementary sequence.

In order to aid the detection and the manipulation of such nucleic acid analogues in diagnostics or other assay procedures and the like operations, it is desirable to provide the nucleic acid analogues with detectable labels. It is also desirable to find ways of capturing said nucleic acid analogues to solid supports. Various labels are described in WO 92/20703. Also, the capture of the nucleic acid analogues to solid supports via bound nucleic acid or nucleic acid analogue sequences acting as capture probes is described.

However, it is desirable to find alternative capture methods and in particular methods which do not require a tailored capture probe which is sequence specific but rather are generally applicable to such nucleic acid analogues.

Furthermore, whilst natural nucleic acids are readily and routinely concentrated by precipitation from solution by ethanol, centrifugation and resuspension, no such convenient method presently exists to aid those working with these nucleic acid analogues.

The present invention now provides according to a first aspect thereof a nucleic acid analogue comprising a polymeric strand which includes a sequence of ligands bound to a backbone made up of linked backbone moieties, which analogue is capable of hybridisation to a nucleic acid of complementary sequence, further comprising, preferably at one terminus of said backbone a chelating moiety capable of binding at least one metal ion by chelation.

Preferably, the backbone is a polyamide, polythioamide, polysulphinamide or polysulphonamide backbone and preferably said chelating moiety is present at the N-terminus.

The chelating moiety preferably comprises a sequence of peptide bonded amino acids.

Preferred sequences of amino acids for use as chelating moieties are -His, Gly,Asp or $-(His)_n$, where n = 3 to 10, e.g. 5 or 6. The longer sequences may bind more than one metal ion per molecule of nucleic acid analogue.

Alternatively, said chelating moiety may be a polycarboxylic acid substituted amine such as ethylenediaminetetraacetic acid (EDTA) or aminotriacetic acid (NTA) and the like.

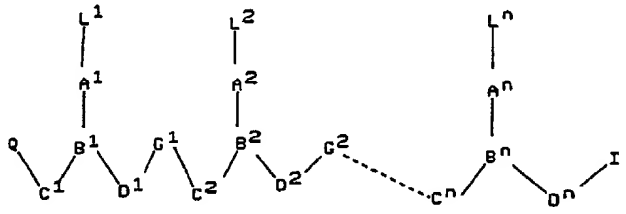
The nucleic acid analogue is preferably capable of hybridising to a nucleic acid of complementary sequence to form a hybrid which is more stable against denaturation by heat than a hybrid between the conventional deoxyribonucleotide corresponding in sequence to said analogue and said nucleic acid.

Said nucleic acid analogue is preferably a peptide nucleic acid in which said backbone is a polyamide backbone, each said ligand being bonded directly or indirectly to a nitrogen atom in said backbone, and said ligand bearing nitrogen atoms mainly being separated from one another in said backbone by from 4 to 8 intervening atoms.

The analogue is preferably capable of hybridising to a double stranded nucleic acid in which one strand has a .

sequence complementary to said analogue, in such a way as to displace the other strand from said one strand.

More preferred PNA compounds for use in the invention have the formula:



Formula 1

n is at least 2,

each of L^1-L^n is independently selected from the group consisting of hydrogen, hydroxy, (C_1-C_4)alkanoyl, naturally occurring nucleobases, non-naturally occurring nucleobases, aromatic moieties, DNA intercalators, nucleobase-binding groups, heterocyclic moieties, reporter ligands and chelating moieties;

each of C^1-C^n is $(CR^6R^7)_y$ (preferably CR^6R^7, CHR^6CHR^7 or $CR^6R^7CH_2$) where R^6 is hydrogen and R^7 is selected from the group consisting of the side chains of naturally occurring alpha amino acids, or R^6 and R^7 are independently selected from the group consisting of hydrogen, (C_2-C_6)alkyl, aryl, aralkyl, heteroaryl, hydroxy, (C_1-C_6)alkoxy, (C_1-C_6)alkylthio, NR^3R^4 and SR^5, where R^3 and R^4 are as defined below, and R^5 is hydrogen, (C_1-C_6)alkyl, hydroxy, alkoxy, or alkylthio-substituted (C_1 to C_6)alkyl or R^6 and R^7 taken together complete an alicyclic or heterocyclic system;

each of D^1-D^n is $(CR^6R^7)_z$ (preferably CR^6R^7, CHR^6CHR^7 or $CH_2CR^6R^7$) where R^6 and R^7 are as defined above;

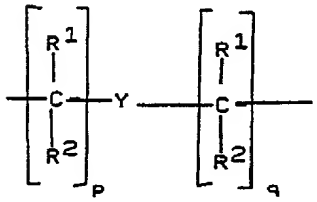
each of y and z is zero or an integer from 1 to 10, the sum y + z being at least 2, preferably greater than 2, but not more than 10;

each of G^1-G^{n-1} is $-NR^3CO-$, $-NR^3C^5-$, $-NR^3SO-$ or $-NR^3SO_2-$, in either orientation, where R^3 is as defined below;

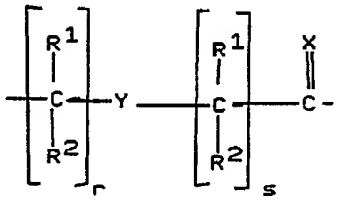
each of A^1-A^n and B^1-B^n are selected such that:

(a) A is a group of formula (IIa), (IIb), (IIc) or (IId), and B is N or R^3N^+; or

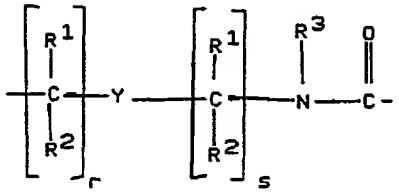
(b) A is a group of formula (IId) and B is CH;



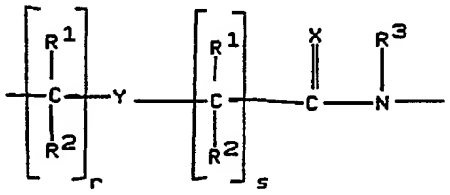
Formula 2



Formula IIb



Formula IIc



Formula IId

wherein:

X is O, S, Se, NR^3, CH_2 or $C(CH_3)_2$;

Y is a single bond, O, S or NR^4;

each of p and q is zero or an integer from 1 to 5, the sum p+q being not more than 10;

each of r and s is zero or an integer from 1 to 5, the sum r+s being not more than 10;

each R^1 and R^2 is independently selected from the group consisting of hydrogen, (C_1-C_4)alkyl which may be hydroxy- or alkoxy- or alkylthio-substituted, hydroxy, alkoxy, alkylthio, amino and halogen; and

each R^3 and R^4 is independently selected from the group consisting of hydrogen, (c_1-C_4)alkyl, hydroxy- or alkoxy- or alkylthio-substituted (C_1-C_4)alkyl, hydroxy, alkoxy, alkylthio and amino;

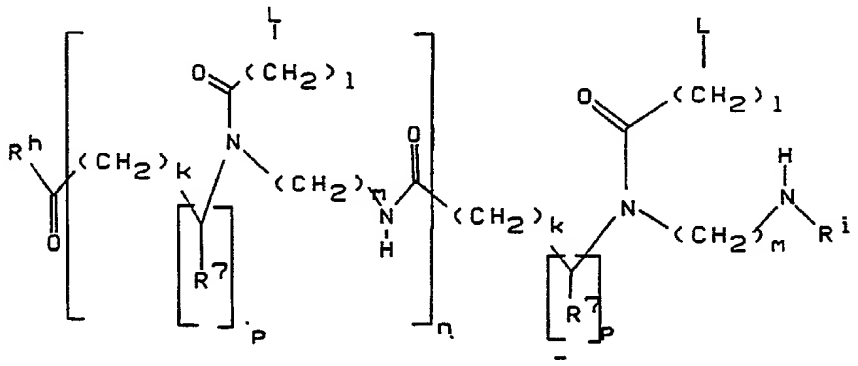
Q is $-CO_2H$, $-CONR'R''$, $-SO_3H$ or $-SO_2NR'R''$ or an activated derivative of $-CO_2H$ or $-SO_3H$; and

I is $-NR'R'''$, where R' and R'' are independently selected from the group consisting of hydrogen, alkyl, amino protecting groups, reporter ligands, intercalators, chelators, peptides, proteins, carbohydrates, lipids, steroids, nucleosides, nucleotides, nucleotide diphosphates, nucleotide triphosphates, oligonucleotides, including both oligoribonucleotides and oligodeoxyribonucleotides, oligonucleosides and soluble and non-soluble polymers, and -R''' is a chelating moiety. "Oligonucleosides" includes nucleobases bonded to ribose and connected via a backbone other than the normal phosphate backbone of nucleic acids.

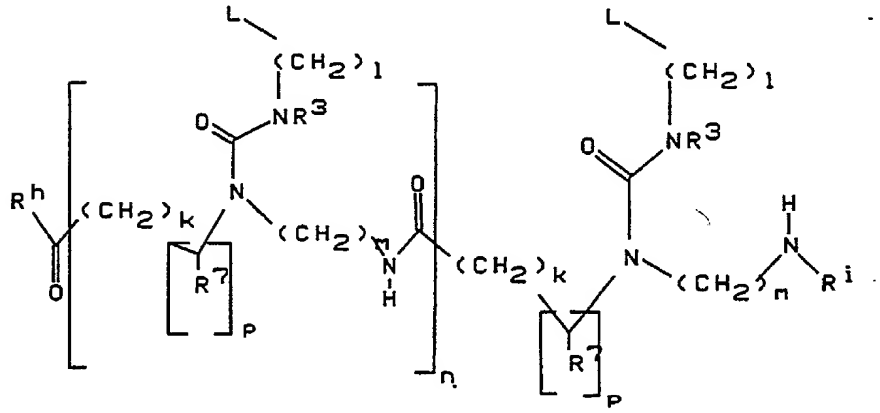
In the above structures wherein R' or R'' is an oligonucleotides or oligonucleosides, such structures can be considered chimeric structures between PNA compounds and the oligonucleotide or oligonucleoside.

Generally, at least one of L^1-L^n will be a naturally occurring nucleobase, a non-naturally occurring nucleobase, a DNA intercalator, or a nucleobase binding group.

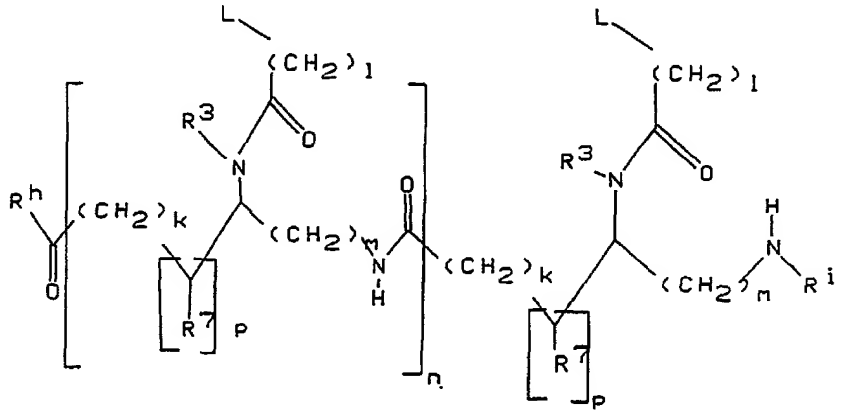
Preferred PNA-containing compounds useful to effect binding to RNA, ssDNA and dsDNA and to form triplexing structures are compounds of the formula III, IV or V:



Formula III



Formula IV



Formula V

wherein:

each L is independently selected from the group consisting of hydrogen, phenyl, heterocyclic moieties, naturally occurring nucleobases, and non-naturally occurring nucleobases;

each R^7 is independently selected from the group consisting of hydrogen and the side chains of naturally occurring alpha amino acids;

n is an integer greater than 1,

each k, l, and m is, independently, zero or an integer from 1 to 5;

each p is zero or 1;

R^h is OH, NH_2 or -NHLysNH$_2$; and

R' is a chelating moiety.

The invention includes according to a second aspect thereof a method of capturing a nucleic acid analogue of the kind described above, which method comprises exposing the

nucleic acid analogue to a solid support bearing chelatable metal ions bonded thereto under conditions such that the chelating moiety of the nucleic acid analogue chelates the said bound metal ions, so capturing the nucleic acid analogue to the solid support.

Alternatively, the capture process may comprise exposing the nucleic acid analogue and chelatable metal ions to a solid support capable of binding the metal ions under conditions such that the metal ions become bound to the solid support and chelated by the chelating moiety of the nucleic acid analogue. The metal ions can if preferred be chelated by the nucleic acid analogue or to the solid support prior to the nucleic acid analogue and the solid support being exposed to one another.

The solid support may comprise a chelating agent such as NTA or EDTA bound thereto chelating ions such as nickel or copper ions which are further chelatable by said nucleic acid analogue.

A particularly preferred solid support is agarose gel and the solid support bearing chelatable metal ions may preferably be Ni-NTA-agarose. Conveniently, the gel may be in a column through which a solution containing the nucleic acid analogue to be captured may be passed, eg. a spin column through which said solution is centrifuged. Another preferred form of solid support is magnetic particles with a surface bearing chelatable metal ions, which may be held thereon by chelating agents as described above.

Such a method preferably comprises capturing said nucleic acid analogue from a first volume of solution by a method as described, removing the solid support and captured nucleic acid analogue from said solution and eluting the nucleic acid analogue from the solid support in a quantity of liquid such as to produce a second volume of a solution of said nucleic acid analogue which is less than said first volume of solution. The nucleic acid analogue is thereby concentrated with respect to its starting solution concentration. The

elution may be carried out with an excess of chelating agent such as EDTA.

A solid support having a nucleic acid analogue bound thereto or capable of capturing such a nucleic acid analogue by the techniques described above may be used to capture from solution a nucleic acid of complementary sequence. A particular virtue of this technique is that one then has the option of removing the captured nucleic acid from the solid support either with or without the nucleic acid analogue.

Thus by treating the system with an excess of a chelating agent such as EDTA, the chelated metal can be removed, so freeing the nucleic acid analogue, and any hybridised nucleic acid. Alternatively, one may liberate the nucleic acid from the nucleic acid analogue on the support by heat or other denaturing methods.

One example of such capture of a nucleic acid would be to hybridise a nucleic acid to the nucleic acid analogue capture probe bearing a chelating moiety, then or previously to chelate metal ions the chelating moiety, and then to capture the resulting complex to a solid bearing chelating groups.

The invention includes in a third aspect thereof a labelled nucleic acid analogue comprising a nucleic acid analogue according to the first aspect of the invention, having chelated thereto via said chelating moiety a metal ion as label or having a labelling moiety linked thereto via a metal ion chelated by said chelating moiety.

Said metal ion is preferably a radio label such as ^{111}indium or ^{99}technetium or a fluorescent label such as europium or terbium.

Nucleic acid analogues according to the first aspect of the invention may be prepared by first synthesizing a PNA by the solid phase techniques described in WO92/20703 to produce a Boc-terminated PNA bound to a solid support at its carboxy end. The PNA may then be extended by removal of the Boc-group to yield a starting point for a standard boc type or

Fmoc type solid phase peptide synthesis adding for instance the required chelating amino acids via the linker 6-amino-hexanoic acid. The protection groups may then be removed and the product may be cleaved from the resin by the Low-High TFMSA procedure. The raw product may be purified by preparative HPLC (suitable conditions being: reversed phase C_{18} eluting with a gradient of A: 0.1% TFA in water and B: 0.1% TFA, 10% water, 89% acetonitrile).

The invention will be illustrated by the following examples making reference to the accompanying drawing in which:

Figure 1 is a bar graph showing the radioactivity counts obtained in the measurements described in Example 1.

In the following Examples, the PNA used has an amimo-ethylglycine backbone and is prepared by the methods specifically described in WO92/20703. The nomenclature used there in respect of PNA's is used here also.

Example 1

Selective purification of DNA by immobilised histidine tagged PNA

The PNA:

Boc-NH-TG(Z)T.A(Z)C(Z)G(Z).TC(Z)A(Z).C(Z)A(Z)A(Z).C(Z)TA(Z)-CONH-Resin

was constructed. This was extended to form the tagged PNA:

$H\text{-}His_5\text{-}NH(CH_2)_5CONH\text{-}TGTACGTCACAACTA\text{-}NH_2$

as follows:

The protected PNA on MBHA resin was coupled with the 6-amino-hexanoic acid linker by boc type solid phase synthesis.

After boc deprotection of the amino terminus, the His_5 motif was built up using an Fmoc strategy. Fmoc-His(Trt)-OH was coupled 2 x 1 hr with diisopropylcarbodiimide in DCM/DMF. The Fmoc group was cleaved by treatment with 20% piperidine in DMF (1 x 5 min and 1 x 10 min). Coupling and Fmoc deprotection were repeated another four times. The trityl protection groups were removed by 50% TFA in DCM (2 x 30 min). Finally the Z groups were removed and the product cleaved from resin by standard HP procedure. The raw product was purified by preparative HPLC.

The tagged PNA was incubated with either complementary or non-complementary, ^{32}P labelled oligonucleotides in a 20μl reaction volume containing 20mM NaH_2PO_4 (pH 8.0) 0.5M NaCl. Incubation was carried out at room temperature for 15 min. At the end of the incubation period 180μl of buffer 1 (20mM NaH_2PO_4 (pH 8.0) 0.5M NaCl) was added and the reaction mixture was loaded onto a Duraphore 0.22μM spin column (Millipore) packed with 200 μl Ni-NTA-agarose (Pharmacia). The column was centrifuged for 30 seconds at 1000 rpm and the radio activity in the flow through (named Sup) was counted using a Geiger Muller tube.

The column was washed three times with 200 μl of buffer 1, and the radioactivity in the flow through (named: Wash I-IV) was counted. The column was loaded with 200μl of buffer 1, incubated at 95^{o}C for 5 min., and centrifuged for 30 seconds at 1000 rpm. The radioactivity in the flow through (named EluI-II) was counted.

The results are shown in Figure 1. As shown, the non-complementary oligonucleotides (black bars) are all lost from the column during the initial washing steps whereas the complementary oligonucleotide (white bars) remains on the column until the binding to the PNA is broken by heat denaturation. Thus, it is shown that PNA carrying a His_5 tag can function as an effective tool in the purification of nucleic acids containing complementary targets.

Example 2

Europium labelling of PNA by chelation

A PNA oligomer ("oligomer 1") was constructed having the sequence:-

Ado - TGT.ACG.TCACAACTA

where "Ado" is the linker 8-amino-3,6-dioxa-octanoic acid linked via its carboxylic acid terminus to the amino terminus of the PNA sequence. The PNA oligomer 1 was built-up on MBHA resin (150 mg, loading:0.1 mmol/g) using the solid phase synthesis methods described in WO92/20703. The product cleaved from the resin. The PNA oligomer 1 (0.1 mg) was dissolved in 1 ml 50 mM $NaHCO_3$ buffer of pH 8.3 containing 0.9% NaCl and mixed with 0.2 mg of the europium salt of N-(4-isothiocyanate phenyl)-methyl-diethylene-triamine-N,N',N'', N'', tetra acetic acid. The labelling reaction was allowed to proceed for 16 hours at ambient temperatures and the product was purified by gel chromatography (G-25).

Example 3

Alternative Europium labelling procedure

PNA-oligomer 1 was made as described in Example 2 but prior to cleavage from the resin the terminal amino group of the ado-linker was Boc deprotected with trifluoracetic acid and then coupled with diethylene triamine pentaacetic acid dianhydride (200 mg in 25 ml DMF). The product was cleaved from the resin by the standard TFMSA procedure and purified by reversed phase HPLC. The product was dissolved in water (1 mg/ml) and added to a 10 mM solution of europium chloride in water to form its europium complex. Excess europium chloride was removed by filtration through a Sephadex G-25 column.

CLAIMS

1. A nucleic acid analogue comprising a polymeric strand which includes a sequence of ligands bound to a backbone made up of linked backbone moieties, which analogue is capable of hybridisation to a nucleic acid of complementary sequence, further comprising at a chelating moiety capable of binding at least one metal ion by chelation.

2. A nucleic acid analogue as claimed in claim 1, wherein the backbone is a polyamide, polythioamide, polysulphinamide or polysulphonamide backbone.

3. A nucleic acid analogue as claimed in claim 2, wherein said linked backbone moieties are peptide bonded amino acid moieties.

4. A nucleic acid analogue as claimed in claim 2 or claim 3, wherein said chelating moiety is present at the N-terminus.

5. A nucleic acid analogue as claimed in any preceding claim, wherein the chelating moiety comprises a sequence of peptide bonded amino acids.

6. A nucleic acid analogue as claimed in claim 2, wherein said sequence of amino acids is -His, Gly,Asp or $-(His)_5$.

7. A nucleic acid analogue as claimed in claim 1, wherein said chelating moiety is a carboxylic acid poly substituted amine.

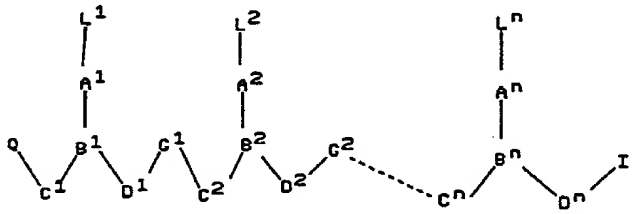
8. A nucleic acid analogue as claimed in any preceding claim, wherein the nucleic acid analogue is capable of hybridising to a nucleic acid of complementary sequence to form a hybrid which is more stable against denaturation by heat than a hybrid between the conventional deoxyribo-

nucleotide corresponding in sequence to said analogue and said nucleic acid.

9. A nucleic acid analogue as claimed in any preceding claim, wherein said nucleic acid analogue is a peptide nucleic acid in which said backbone is a polyamide backbone, each said ligand being bonded directly or indirectly to an aza nitrogen atom in said backbone, and said ligand bearing nitrogen atoms mainly being separated from one another in said backbone by from 4 to 8 intervening atoms.

10. A nucleic acid analogue as claimed in any preceding claim, wherein the analogue is capable of hybridising to a double stranded nucleic acid in which one strand has a sequence complementary to said analogue, in such a way as to displace the other strand from said one strand.

11. A nucleic acid analogue as claimed in any preceding claim, wherein the nucleic acid analogue has the general formula 1:



Formula 1

wherein:

n is at least 2,

each of L^1-L^n is independently selected from the group consisting of hydrogen, hydroxy, (C_1-C_4)alkanoyl, naturally

occurring nucleobases, non-naturally occurring nucleobases, aromatic moieties, DNA intercalators, nucleobase-binding groups, heterocyclic moieties, reporter ligands and chelating moieties;

each of C^1-C^n is $(CR^6R^7)_y$ where R^6 is hydrogen and R^7 is selected from the group consisting of the side chains of naturally occurring alpha amino acids, or R^6 and R^7 are independently selected from the group consisting of hydrogen, $(C_2$-$C_6)$alkyl, aryl, aralkyl, heteroaryl, hydroxy, $(C_1$-$C_6)$alkoxy, $(C_1$-$C_6)$alkylthio, NR^3R^4 and SR^5, where R^3 and R^4 are as defined below, and R^5 is hydrogen, $(C_1$-$C_6)$alkyl, hydroxy, alkoxy, or alkylthio-substituted $(C_1$ to $C_6)$alkyl or R^6 and R^7 taken together complete an alicyclic or heterocyclic system;

each of D^1-D^n is $(CR^6R^7)_z$ where R^6 and R^7 are as defined above;

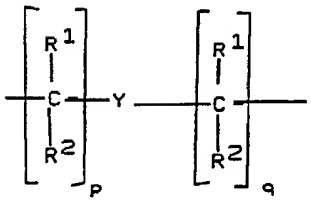
each of y and z is zero or an integer from 1 to 10, the sum y + z being from 2 to 10;

each of G^1-G^{n-1} is $-NR^3CO-$, $-NR^3C^5-$, $-NR^3SO-$ or $-NR^3SO_2-$, in either orientation, where R^3 is as defined below;

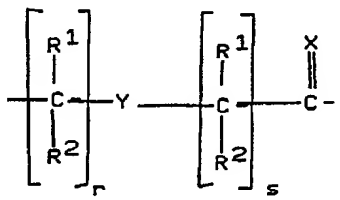
each of A^1-A^n and B^1-B^n are selected such that:

(a) A is a group of formula (IIa), (IIb), (IIc) or (IId), and B is N or R^3N^+; or

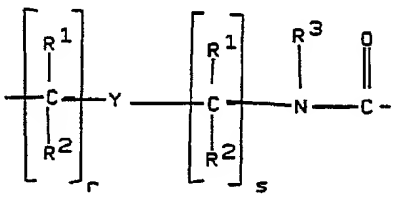
(b) A is a group of formula (IId) and B is CH;



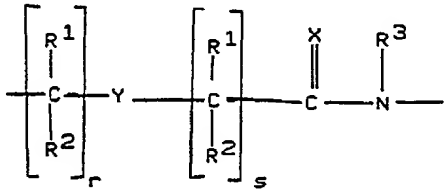
Formula 10



Formula IIb



Formula IIc



Formula IId

wherein:

X is O, S, Se, NR^3, CH_2 or $C(CH_3)_2$;

Y is a single bond, O, S or NR^4;

each of p and q is zero or an integer from 1 to 5, the sum p+q being not more than 10;

each of r and s is zero or an integer from 1 to 5, the sum r+s being not more than 10;

each R^1 and R^2 is independently selected from the group consisting of hydrogen, (C_1-C_4)alkyl which may be hydroxy- or alkoxy- or alkylthio-substituted, hydroxy, alkoxy, alkylthio, amino and halogen; and

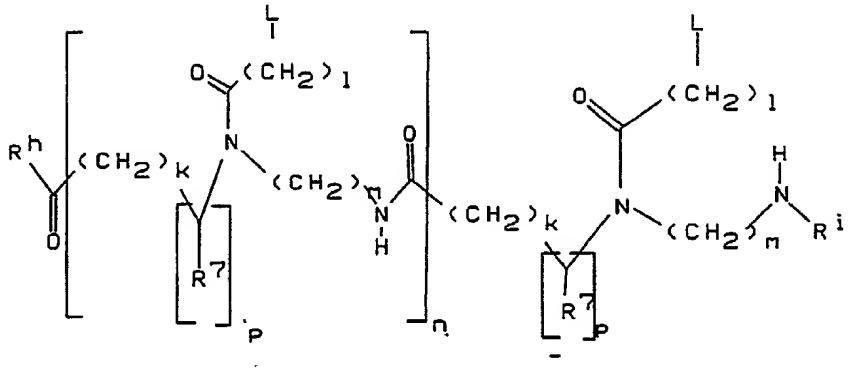
each R^3 and R^4 is independently selected from the group consisting of hydrogen, (c_1-C_4)alkyl, hydroxy- or alkoxy- or alkylthio-substituted (C_1-C_4)alkyl, hydroxy, alkoxy, alkylthio and amino;

Q is $-CO_2H$, $-CONR'R''$, $-SO_3H$ or $-SO_2NR'R''$ or an activated derivative of $-CO_2H$ or $-SO_3H$; and

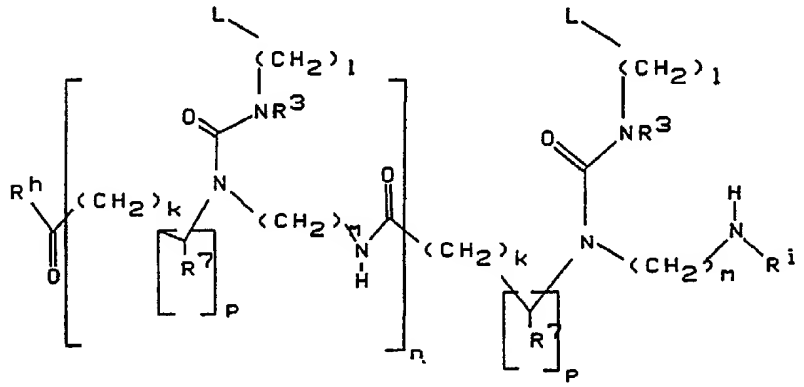
I is $-NR'R'''$, where R' and R'' are independently selected from the group consisting of hydrogen, alkyl, amino protecting groups, reporter ligands, intercalators, chelators, peptides, proteins, carbohydrates, lipids, steroids, nucleosides, nucleotides, nucleotide diphosphates, nucleotide triphosphates, oligonucleotides, including both oligoribo-

nucleotides and oligodeoxyribonucleotides, oligonucleosides and soluble and non-soluble polymers, and -R''' is a chelating moiety.

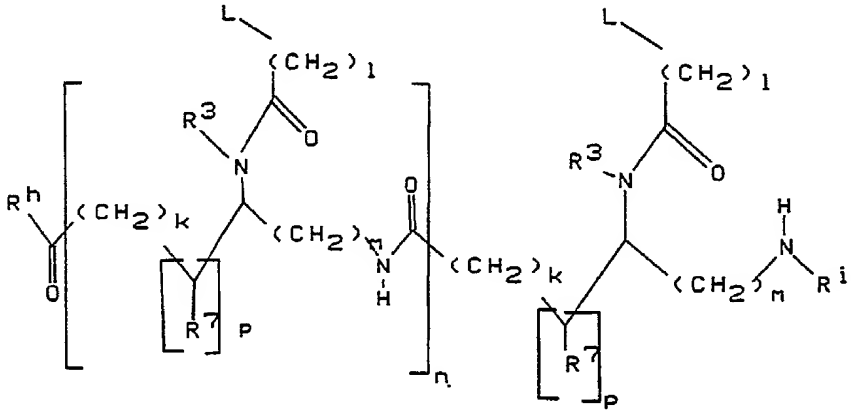
12. A nucleic acid analogue as claimed in Claim 11, wherein said nucleic acid analogue comprises a compound of the general formula III, IV or V:



Formula III



Formula IV



Formula V

wherein:

each L is independently selected from the group consisting of hydrogen, phenyl, heterocyclic moieties, naturally occurring nucleobases, and non-naturally occurring nucleobases;

each R^7 is independently selected from the group consisting of hydrogen and the side chains of naturally occurring alpha amino acids;

n is an integer greater than 1,

each k, l, and m is, independently, zero or an integer from 1 to 5;

each p is zero or 1;

R^h is OH, NH_2 or -NHLysNH$_2$; and

R^i is a chelating moiety.

13. A nucleic acid analogue bearing a chelating moiety substantially as described in any one of Examples 1 to 3.

14. A method of capturing a nucleic acid analogue according to any preceding claim, which method comprises exposing the

nucleic acid analogue to a solid support bearing chelatable metal ions bonded thereto under conditions such that the chelating moiety of the nucleic acid analogue chelates the said bound metal ions, so capturing the nucleic acid analogue to the solid support.

15. A method as claimed in claim 14, wherein the solid support comprises NTA or EDTA bound thereto chelating nickel or copper ions which are further chelatable by said nucleic acid analogue.

16. A method of concentrating a solution of a nucleic acid analogue according to any one of claims 1 to 13, comprising capturing said nucleic acid analogue from a first volume of solution by a method as claimed in claim 14 or claim 15, removing the solid support and captured nucleic acid from said solution and eluting the nucleic acid analogue from the solid support in a quantity of liquid such as to produce a second volume of a solution of said nucleic acid analogue which is less than said first volume of solution.

17. A labelled nucleic acid analogue comprising a nucleic acid analogue as claimed in any one of claims 1 to 13, having chelated thereto via said chelating moiety a metal ion as label or having a labelling moiety linked thereto via a metal ion chelated by said chelating moiety.

18. A labelled nucleic acid analogue as claimed in claim 17, wherein said metal ion is a radio label or a fluorescent label.

19. A labelled nucleic acid substantially as hereinbefore described in Example 2 or Example 3.

20. A method of capturing a nucleic acid substantially as hereinbefore described in Example 1.

20

Patents Act 1977
Examiner's report to the Comptroller under Section 17
(The Search report)

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Relevant Technical Fields

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MR C SHERRINGTON

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13 FEBRUARY 1995

Databases (see below)
(i) UK Patent Office collections of GB, EP, WO and US patent specifications.

(ii) ONLINE DATABASES: WPI, CLAIMS

Documents considered relevant following a search in respect of Claims :-
1-20

Categories of documents

X: Document indicating lack of novelty or of inventive step.

Y: Document indicating lack of inventive step if combined with one or more other documents of the same category.

A: Document indicating technological background and/or state of the art.

P: Document published on or after the declared priority date but before the filing date of the present application.

E: Patent document published on or after, but with priority date earlier than, the filing date of the present application.

&: Member of the same patent family; corresponding document.

Category	Identity of document and relevant passages		Relevant to claim(s)
P,X	WO 94/25477 A1	(NIELSON, PETER E ET AL) especially page 6, line 23 to page 7, line 3; Claim 1, particularly page 87, line 6	1 (at least)
A	WO 93/24511 A1	(HOWARD FLOREY INSTITUTE OF EXPERIMENTAL PHYSIOLOGY AND MEDICINE) whole document	1
X	WO 92/20703 A1	(BUCHARDT, OLE ET AL) whole document, especially Examples 52 to 63; Claim 1, especially page 109, line 20	1, 6 (at least)
A	Science 1991, 254, 1497-1500 Sequence-Sleective Recognition of DNA by Strand Displacement with Thymine- Substituted		1
A	J. Am. Chem. Soc. 1992, 114(5), 1895-1897 Peptide Nucleic Acids (PNA) Oligonucleatide Analogues with an Achiral Peptide Backbone		1
A	Proc. Natl. Acad. Sci. USA 1993,90(5), 1667-1670 DNA unwinding upon strand-displacement binding of a thymini-substituted polyamide to double-stranded DNA		1

Databases: The UK Patent Office database comprises classified collections of GB, EP, WO and US patent specifications as outlined periodically in the Official Journal (Patents). The on-line databases considered for search are also listed periodically in the Official Journal (Patents).